

## Equine Zygomycosis Caused by *Conidiobolus lamprauges*

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Received 29 March 1988/Accepted 2 December 1988

**A 15-year-old Arabian mare from southern Louisiana with a 2-month history of periodic epistaxis and severe weight loss had a large, fibrosing, granulomatous mass containing numerous nodules ("kunkers") projecting dorsally into the nasopharynx, and was euthanized at the owner's request. In addition to these kunkers, the mass contained a single trematode tentatively identified as *Fasciola hepatica*. Several kunkers were removed, washed thoroughly in sterile water and embedded in nutrient agars; the fungus that grew out of them was identified as *Conidiobolus lamprauges* Drechsler (Entomophthorales: Ancylistaceae). This is the first report of *C. lamprauges* from any vertebrate mycosis, and only the third *Conidiobolus* species reported from vertebrates. Unlike many other entomophthoraleans, the fungus isolated from this mycosis grew well at 37°C. The possible means by which *Conidiobolus* species may infect vertebrates is discussed. These mycoses probably result most often from chronic exposure during sleep to conidia discharged from fungal growth on decaying plant material in the bedding.**

Zygomycoses of horses are caused by species in two genera of the Entomophthorales (Zygomycetes), *Conidiobolus* and *Basidiobolus*. Entomophthoralean mycoses of horses are usually caused by *Conidiobolus coronatus* (*Entomophthora coronata*) and are uncommon diseases characterized by ulcerative, granulomatous lesions involving the nasofacial area, especially the nostril (3). Although entomophthoralean fungi are best known as obligate pathogens of insects and other arthropods, most *Conidiobolus* species are known only as saprobes; only a few *Conidiobolus* species are weakly facultative or obligate entomopathogens.

Mycoses of humans caused by *Conidiobolus coronatus* nearly always take the form of a painless, spreading induration affecting the subcutis overlaying the paranasal sinuses. Prolonged development without successful surgical or chemotherapeutic treatment may result in considerable destruction of tissue and bone adjacent to the affected tissues. The fungus may be detected in the proximal lymph nodes (18). These relatively uncommon infections have been reported from many tropical and subtropical regions, but seem to be most frequent in Africa and India (1, 29).

*Conidiobolus* infections of equines are less common than those from humans, but also tend to be restricted to warm climates. Infections by *C. coronatus* have been seen in the southern United States (3, 9), Costa Rica (21), Colombia (28), Brazil (17), Australia (16, 24, 26), and India (5). *Conidiobolus* mycoses of other nonhuman mammals are much rarer but have been diagnosed in an East African chimpanzee (30) and, apparently, a mandrill (23) and dolphin (22); the latter two cases were not confirmed with cultures from the affected tissues.

With the sole exceptions of the recoveries of *Conidiobolus incongruus* Drechsler from a mediastinal mass in a 15-month-old boy from West Virginia (10, 20) and from a fatal disseminated mycosis in a 20-year-old female in Thailand (4), all cultures previously isolated from vertebrate conidiobolomycoses have been identified as *C. coronatus*. This report

describes an unusual zygomycotic infection of a horse caused by *Conidiobolus lamprauges* Drechsler and is the first record of this species causing disease in any vertebrate.

**Clinical findings and necropsy.** A 15-year-old, 410-kg Arabian mare stabled near Baton Rouge, La., presented with a 2-month history of periodic epistaxis and severe weight loss. By endoscopy, a large protruding mass containing numerous yellow, cylindrical, corallike concretions ("kunkers") was visualized in the caudal portion of the nasopharynx. Because of poor prognosis, the horse was euthanized at the owner's request.

Postmortem examination revealed an 8-cm-diameter nodule incorporated within and projecting dorsally from the rear of the soft palate and bilaterally encroaching upon the pharyngeal opening of the guttural pouches (Fig. 1, panel 1). The nodule was diffusely roughened; both exterior and cut surfaces contained numerous kunkers measuring approximately 0.2 cm in diameter and 0.5 cm in length. Both guttural pouches were partially filled with a serosanguinous fluid containing many kunkers.

Microscopic examination revealed the pharyngeal mass to be composed of a fibrosing, granulomatous inflammation with multifocal hemorrhages and necrosis and a predominance of eosinophils. Scattered throughout were fragments of thin-walled hyphae 6 to 10  $\mu$ m in diameter; these hyphae were occasionally branched (Fig. 1, panel 2) and infrequently septate (Fig. 1, panel 3) and were surrounded by the prominent granular eosinophilic (Splendore-Hoeppli) precipitate (Fig. 1, panel 4) that is a constant characteristic of entomophthoralean mycoses of mammals. As is frequently observed with entomophthoralean fungi, many hyphae in the tissue mass appeared to be empty tubes devoid of significant cytoplasmic contents (Fig. 1, panel 3).

An oblique section of a trematode was present in a section from the center of the mass. The trematode contained operculate eggs, sperm within a sperm duct, prominent, darkly pigmented cecal epithelial cells, and a cuticle with many short spines. These characters suggested that this trematode was the liver fluke, *Fasciola hepatica*, although no definitive identification was possible because very little of

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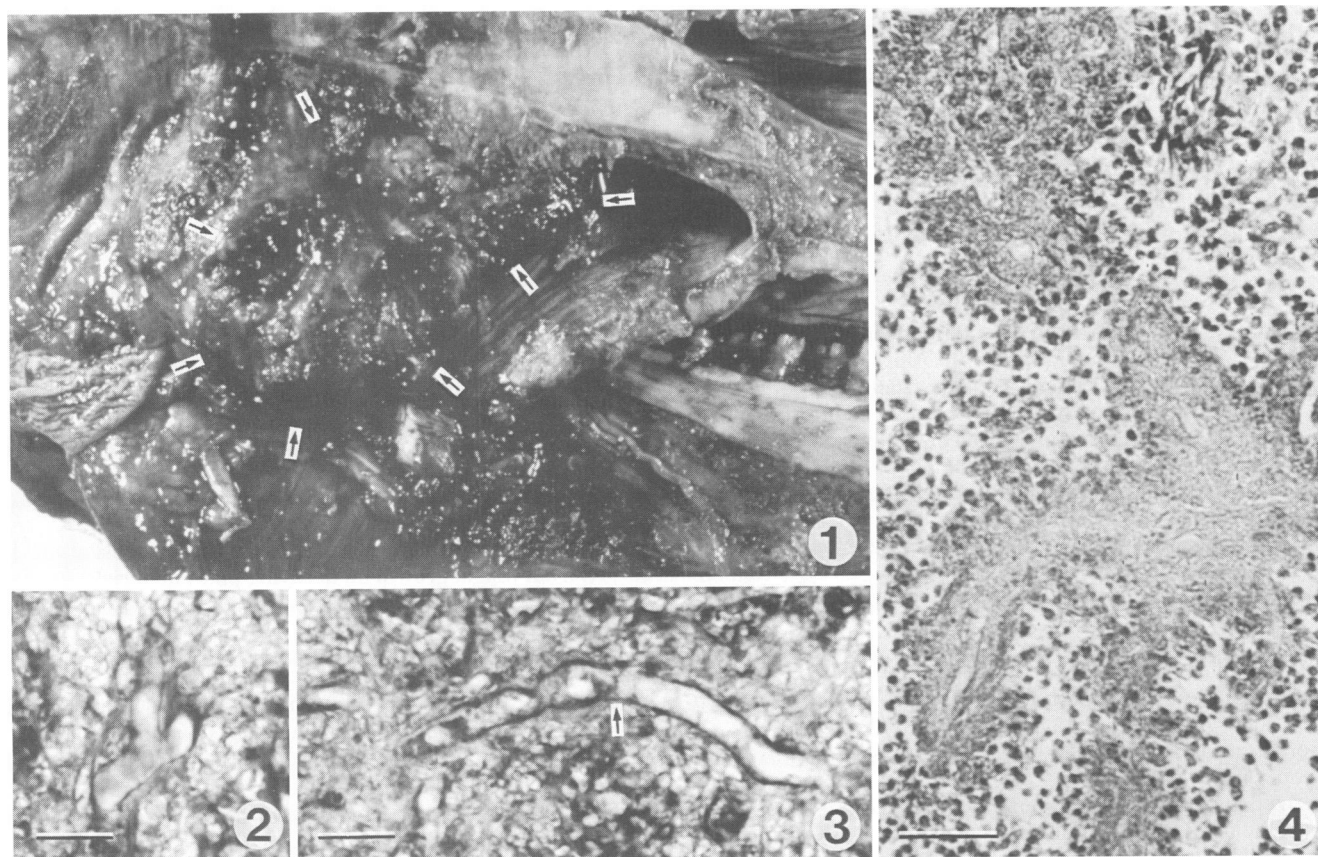


FIG. 1. (Panel 1) Granulomatous nasopharyngeal nodule caused by *Conidiobolus lamprauges*. Arrows indicate the margin of the mycotized mass; photograph is about one-half the original size. A branched cell (panel 2) and a hypha with a cytoplasm-filled cell separated from an empty portion of the hypha by a septum (panel 3, arrow) are also shown; large portions of hyphae in the tissue mass were emptied of cytoplasmic contents. Gomori methenamine-silver counterstained by hematoxylin and eosin; bar, 20  $\mu\text{m}$ . (Panel 4) Hyphae surrounded by eosinophilic granular precipitate, the so-called Splendore-Hoeppli phenomenon. Hematoxylin and eosin stained; bar, 40  $\mu\text{m}$ .

the fluke remained in the paraffin block and the remainder was not found in the wet tissues.

The mucosa of the guttural pouch was multifocally eroded and proliferative with a modest, diffuse infiltration of lymphocytes, plasma cells, and macrophages in the submucosa. In the anteroventral portions of the lung, the alveoli and bronchioles were filled with proteinaceous fluid and neutrophils. Many bronchioles also contained clumps of bacteria and, occasionally, plant material.

**Isolation and characterization of the fungus.** Several kunkers were washed six times in sterile water and partially embedded into Sabouraud dextrose and vegetable extract agars. Fungal growth from the tissue was evident within 24 h, and abundant spores were noted after 7 days.

Colonies growing on Sabouraud dextrose agar-1% yeast extract (SDAY) or on potato dextrose agar (PDA) were opaque and were flat for the first several days but became tightly wrinkled after about 10 days. The average diameters of colonies grown in the dark for 3 and 6 days at 22°C on SDAY were 18.6 and 37.8 mm, respectively, and on PDA were 17.1 and 38.4 mm, respectively. The hyphae were predominantly unbranched and 4.8 to 8.0  $\mu\text{m}$  in diameter. Some hyphae toward the edge of a colony bore many short, knobby branches. Hyphal growth and conidial production were more prolific on SDAY than on PDA. Aerial mycelium was sparse and production of innumerable zygospores began early on PDA; on SDAY, aerial mycelium and conidial

production were more prominent. No conspicuous odor or pigmentation developed on either medium.

This fungus grew well in darkness at 37°C. The average diameters of colonies on SDAY after 24 and 48 h were 21 and 42 mm, respectively; on PDA after 24 and 48 h, the average colony diameters were 16 and 29 mm, respectively.

The conidia were globose to obovoid with a rounded to apiculate basal papilla. In what was probably a mixed population of primary and secondary conidia discharged from a colony growing on SDAY, the overall length (including the papilla) was  $21.0 \pm 1.7 \mu\text{m}$  (mean plus or minus standard deviation;  $n = 50$ ; measured in lactophenol-aniline blue). Their diameter was  $17.5 \pm 2.0 \mu\text{m}$ . The length/width ratio was  $1.2 \pm 0.1$  and ranged from 1.0 to 1.4.

The resting spores of *C. lamprauges* formed in PDA were smooth surfaced and globose to subglobose and had an average diameter of  $17.0 \pm 1.2 \mu\text{m}$  (mean plus or minus standard deviation;  $n = 50$ ; measured in lactophenol-aniline blue). The zygospore wall was relatively thin (1 to 3  $\mu\text{m}$  thick), and mature resting spores contained a large, subcentric oil droplet; many zygospores were seen to lie rather loosely in the zygosporangial wall. The zygospores always developed in the axis of one of the parental cells rather than arising as lateral buds from the gametangia; most zygospores arose from fusions of adjacent cells in a hypha, although some diclinous conjugations also occurred.

*Conidiobolus* species are often difficult to identify, but this

particular fungus was readily determined to be *C. lamprauges* Drechsler. Its characters match well with the published descriptions of *C. lamprauges* (8, 19). The culture was accessioned into the U.S. Department of Agriculture-Agricultural Research Service Collections of Entomopathogenic Fungi (Ithaca, N.Y.) as ARSEF 2338 and is available upon request from one of us (R.A.H.).

*Fasciola hepatica*, when present in unusual hosts such as horses, may be found in extrahepatic locations such as in the lungs or under the skin (31). The presence of the fluke within this nasopharyngeal mass may have been due to the increased blood supply to the area. Alternatively (but less probably), the fluke may have lodged in this location first and incited a mucosal inflammation which provided a more favorable site for fungal development. In any event, the major pathology observed here was due to the fungus rather than to the trematode.

The predominance of eosinophils in the inflammatory exudate and the eosinophilic granular (Splendore-Hoeppli) precipitate surrounding hyphae in the case reported here are characteristic of infections by *Conidiobolus* and *Basidiobolus* species as well as those by an oomycete, *Pythium insidiosum*. The morphology of hyphae in the pharyngeal mass could not be distinguished from that of *C. coronatus* even if long hyphal segments were visible in tissue sections.

The nearly all *Conidiobolus* infections in horses (and other vertebrates) occur in the area of the nostrils and adjacent tissues which are subject to evaporative cooling from the skin and/or tidal airflow in the nasal passages. That the nasopharyngeal mycosis reported here is much deeper than usual may be explained, in part, by the fact that the causative fungus grew well at 37°C; the normal body temperature of horses is 37.2 to 37.4°C. Only one other entomophthoralean mycosis from a horse was reported to involve the mouth and pharynx (26).

Little is known of the temperature tolerances of most *Conidiobolus* species, but entomogenous species seem generally unable to survive temperatures  $\geq 35^\circ\text{C}$  (13) whereas some isolates of *C. coronatus* from vertebrates may survive up to 4 days at 42°C (6). *Basidiobolus* species are often found to survive at 37 to 40°C (11, 12, 32), and their infections of vertebrates are generally more deeply seated than those caused by *Conidiobolus* species.

**Etiology of entomophthoralean zygomycosis.** The means by which vertebrates become infected by *Conidiobolus* and *Basidiobolus* species remain uncertain. Mycoses are usually too advanced by the time they receive medical attention to determine how or when the infection began. These infections have been hypothesized to occur either by contact with spores on the ground, by dead and infected insects depositing spores directly on the nostrils, or by inhalation of infected insects (10, 25, 26).

The entomophthoraleans known to infect vertebrates are only rarely (or never) reported to attack insects. *C. coronatus* occurs primarily in plant detritus and soil, but is also known from insects (27); especially in the tropics, this species can be a distressingly common contaminant of dead insects or be a secondary pathogen of hosts already diseased by a more virulent (primary) fungal pathogen. *C. lamprauges* is apparently rare and occurs primarily in plant detritus or soil (19). Nonetheless, King (19) isolated a culture (ATCC 28996) of *C. lamprauges* from an anthomyiid fly patently infected by *Entomophthora muscae*, a virulent pathogen of flies that can be difficult to isolate in vitro. The presence of *C. lamprauges* on the fly is assumed to be have been accidental and fortuitous.

The number of human mycoses caused by *C. coronatus* cannot be accounted for by the inhalation of insects, whose probability of infection by this fungus is, in turn, miniscule. Inhaled insects are more likely to be cleared rapidly from the nasal passages (by sneezing, etc.) or swept through the nasal passages and swallowed rather than to lodge in the nose or sinuses where sporulation from infected insects could cause infection; it is even less probable that conidiobolomycoses could begin with a few spores borne superficially on otherwise healthy insect. Hypotheses about the origins of entomophthoralean mycoses of vertebrates that invoke infected insects are wholly unrealistic in view of the biologies of the fungi and their hosts.

Vertebrate infections caused by *Conidiobolus* and *Basidiobolus* species are rare in view of the extremely wide distributions of these fungi in plant detritus and soil rich in organic matter (7, 19). This suggests that mycoses caused by these saprobes or weak pathogens are not easily acquired and that their hosts were repeatedly challenged or immunologically compromised.

Horses and chimpanzees sleep on beds of vegetable matter that, if damp and beginning to decay, could provide ideal habitats for the growth of *Conidiobolus* species. The conidia of these fungi can be discharged upwards to a height of ca. 2 cm, high enough to be inhaled during sleep or to land in the outer portions of the nasal tract where secondary conidia could form, discharge, and lodge deeper in the nasal passages. The geographical distribution of entomophthoralean mycoses of vertebrates is not coextensive with that of the causative agents; most reported cases from humans and other vertebrates are from subtropical or tropical regions. Human conidiobolomycosis seems to be reported most frequently from regions where straw or other unprocessed vegetable material may be commonly used for bedding.

The hypothesis that most cases of conidiobolomycosis in vertebrates begin with spores lodged in the nose during sleep cannot explain all known or suspected instances of this mycosis. Neither this nor any other suggested mode of infection can be proven, but this new hypothesis is more plausible than any invoking the inhalation of infected insects. If this hypothesis is correct, the mycosis discussed here may have arisen from spores discharged from decaying plant matter in the mare's pasture or stable. By extension, the incidence of equine and human infections by *Conidiobolus* species should be reduced by assuring that bedding is kept as clean and dry as possible.

**Impact of revised entomophthoralean taxonomy on medical mycology.** Recent major changes in entomophthoralean systematics have affected the taxonomy of the medically important species of this order. The genera *Conidiobolus* and *Basidiobolus* are now placed in the families Ancylistaceae and Basidiobolaceae, respectively (2, 15, 15a). In this revised classification, no positive identification of *C. coronatus* is possible unless conidia bearing villose spines (19) are seen, since this species, *Conidiobolus incongruus*, and eight other species in *Conidiobolus* subgenus *Delacroixia* (2, 33) form secondary microconidia.

The family Entomophthoraceae was long regarded to include all genera of this order but is now restricted to obligate entomopathogens (2, 14, 15) whose likelihood of causing vertebrate mycoses is virtually nil. Nonetheless, it should be realized that, in rare and extraordinary circumstances, nearly any fungus might cause an unusual mycosis in a vertebrate.

We thank John B. Malone for help with the trematode identification.

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